AD

TECHNICAL MANUSCRIPT 555

A PLAQUE ASSAY FOR COXIELLA BURNETII

Joseph E. McDade
Peter J. Gerone

SEPTEMBER 1969

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

9

DDC AVAILABILITY NOTICES

Qualified requesters may obtain copies of this publication from DDC.

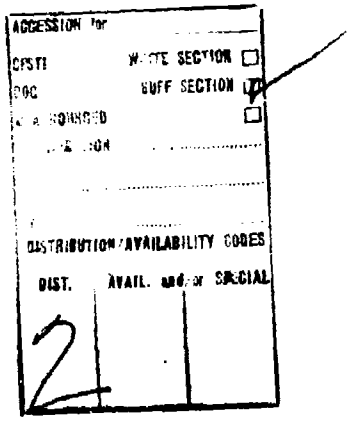
Foreign announcement and dissemination of this publication by DDC is not authorized.

Release or announcement to the public is not authorized.

DISPOSITION INSTRUCTIONS

Destroy this publication when it is no longer needed. Do not return it to the originator.

The findings in this publication are not to be construed as an official Department of the Army position, unless so designated by other authorized documents.



DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland 21701

TECHNICAL MANUSCRIPT 555

A PLAQUE ASSAY FOR COXIELLA BURNETII

Joseph E. McDade

Peter J. Gerone

Virus & Rickettsia Division
BIOLOGICAL SCIENCES LABORATORIES

Project 1B562602A059

September 1969

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

ACKNOWLEDGMENT

We acknowledge the excellent technical assistance of Mr. Orville Brand, who performed the complement-fixation tests.

ABSTRACT

The plaque assay system developed in this laboratory for the typhus, spotted fever, and scrub typhus groups of rickettsiae is also appropriate for Coxiella burnetii.

A PLAQUE ASSAY FOR COXIELLA BURNETII*

In several recent reports,[1-3] we demonstrated that a convenient and sensitive plaque assay system had been developed for the typhus, spotted fever, and scrub typhus groups of rickettsiae. We now report that this same plaque assay procedure has been successfully employed with Coxiella burnetii.

The organism used in this study, the AD strain of C. burnetii, was originally isolated from raw milk in California in 1948.[4] Our working seed consisted of 10% yolk sac suspensions, prepared in brain heart infusion broth (BHIB), of the 10th yolk sac passage of this strain.

The plaque assay procedure used in this study was the same procedure described in more detail in earlier reports.[2,3] Twenty-four-hour chick embryo primary monolayers in 30-ml plastic tissue culture bottles were infected for 15 minutes with serial tenfold dilutions (in BHIB) of C. burnetii. The infected monolayers were then covered with 5 ml of an overlay of medium 199 (5% calf serum) containing agarose at a final concentration of 0.5%. The monolayers were incubated in a closed system at 32 C for 8 to 11 days. Plaques were stained by adding an overlay containing neutral red as described earlier.[3]

The plaque morphology of the AD strain of C. burnetii is shown in Figure 1. This strain produces small (approximately 1 mm), somewhat indistinct plaques, which are morphologically similar to plaques formed by typhus and scrub typhus rickettsiae.[1,2] Although plaques produced by C. burnecii are small, plaque counts are easily determined.

Studies were initiated to compare the plaque titer with the 50% infectious dose (ID_{50}) in mice.[5] Groups of eight 16- to 18-g male Swiss mice (Fort Detrick strain) were injected IP with 0.1 ml of serial tenfold dilutions of C. burnetii suspensions in BHIB ranging from 10^{-6} through 10^{-11}. Twenty-two days later the animals were bled and the complement-fixation titers of the sera were determined using commercially prepared (Lederle) Nine Mile strain of C. burnetii as antigen. Sera having a complement-fixation titer of 1:8 or greater were considered positive. The ID_{50} was then computed by the Spearman-Kärber method of calculation. The plaque titers of three ampoules from the same seed pool were determined in parallel with the ID_{50} determination.

A comparison of the mouse ID_{50} with the plaque titer demonstrated that the ID_{50} assay is more sensitive for C. burnetii than the plaque titration procedure. The seed pool had a mouse ID_{50} titer of 9.75 $\log_{10}$/ml and an average plaque titer of 8.4 $\log_{10}$/ml. There was less than one-tenth log difference among the plaque titers of the three ampoules tested.

---

* This report should not be used as a literature citation in material to be published in the open literature. Readers interested in referencing the information contained herein should contact the senior author to ascertain when and where it may appear in citable form.

FIGURE 1. Plaques Formed by *Coxiella burnetii* on Chick Embryo Primary Monolayers. From left to right, 10^{-6}, and 10^{-7} dilutions of inoculum.

Although the plaque assay procedure for C. burnetii is somewhat less sensitive than the ID_{50} assay, it offers some advantages. The plaque assay is faster (10 days compared with 22), far less laborious, much more economical, and provides the opportunity to isolate organisms from a single focus of infection. For many experimental purposes these advantages may offset the reduced sensitivity of the system.

The results of these and previous tests[1-3] indicate that the plaque assay system developed in this laboratory is a sensitive titration procedure for diverse types of rickettsiae. We have been successful in obtaining plaques with representative species of each of the four groups of these organisms. Moreover, we have not found any rickettsiae that we have not been able to plaque. These findings demonstrate that this assay may be appropriate for all rickettsiae, and should facilitate studies of these organisms.

We are unable to explain why this procedure has been successful where others have failed. In a few preliminary studies we varied several procedures in the assay, but no single factor could be identified as the key to the success or failure of the procedure. Further studies are necessary to elucidate the critical steps in this plaque assay procedure.

LITERATURE CITED

1. McDade, J.E.; Gerone, P.J. September 1969. A plaque assay for Rickettsia tsutsugamushi, (Technical Manuscript 556). Virus and Rickettsia Division, Fort Detrick, Frederick, Maryland.

2. McDade, J.E.; Stakebake, J.R.; Gerone, P.J. 1969. A plaque assay system for several species of Rickettsia. J. Bacteriol. (In press).

3. Weinberg, E.H.; Stakebake, J.R.; Gerone, P.J. 1969. Plaque assay for Rickettsia rickettsi. J. Bacteriol. 98:398-402.

4. Huebner, R.J.; Jellison, W.L.; Beck, M.D.; Parker, R.R.; Shepard, C.C. 1948. Q fever studies in southern California: I. Recovery of Rickettsia burneti from raw milk. Public Health Rep. 63:214-222.

5. Schneider, M.D.; Ehrlich, P.; Yamashiroya, M.; Miller, S. 1966. Quantitative assay of Coxiella burnetii in mice. Appl. Microbiol. 14:767-768.

Unclassified
Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY *(Corporate author)*
Department of the Army
Fort Detrick, Frederick, Maryland, 21701

2a. REPORT SECURITY CLASSIFICATION
Unclassified

2b. GROUP

3. REPORT TITLE
A PLAQUE ASSAY FOR COXIELLA BURNETII

4. DESCRIPTIVE NOTES *(Type of report and inclusive dates)*

5. AUTHOR(S) *(First name, middle initial, last name)*
Joseph E. McDade
Peter J. Gerone

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
September 1969	9	5

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO. 1B562602A059	Technical Manuscript 555
c.	9b. OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)*
d.	CMs 6586

10. DISTRIBUTION STATEMENT
Qualified requesters may obtain copies of this publication from DDC.
Foreign announcement and dissemination of this publication by DDC is not authorized.
Release or announcement to the public is not authorized.

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	Department of the Army Fort Detrick, Frederick, Maryland, 21701

13. ABSTRACT
The plaque assay system developed in this laboratory for the typhus, spotted fever, and scrub typhus groups of rickettsiae is also appropriate for Coxiella burnetii.

14. Key Words

Coxiella burnetii, AD strain
Plaque
Assay
Rickettsiae

DD FORM 1 NOV 68 1473 REPLACES DD FORM 1473, 1 JAN 64, WHICH IS OBSOLETE FOR ARMY USE.

Unclassified
Security Classification